

SHORT REPORT

Outbreak investigation of fever mimicking dengue in Havelock Island, an important tourist destination in the Andaman & Nicobar Archipelago, 2014

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SUMMARY

An upsurge of fever cases of unknown origin, but resembling dengue and leptospirosis was reported in Havelock, Andaman & Nicobar Islands, an important tourism spot, during May 2014. Investigations were carried out to determine the aetiology, and to describe the epidemiology of the outbreak. The data on fever cases attending Primary Health Centre (PHC), Havelock showed that the average number of cases reporting per week over the last 2 years was 46·1 (95% confidence interval 19·4–72·9). A total of 27 (43·5%) patients out of the 62 suspected cases were diagnosed as having DENV infection based on a positive enzyme immunoassay or reverse transcriptase–polymerase chain reaction. The overall attack rate was 9·4 cases/1000 population and it ranged between 2·8 and 18·8/1000 in different villages. The nucleotide sequencing showed that the virus responsible was DENV-3. DENV-3 was first detected in the Andaman & Nicobar Islands in 2013 among wharf workers in Port Blair and within a year it has spread to Havelock Island which is separated from South Andaman by 36 nautical miles.

Key words: Dengue, DENV-3, Havelock Island, outbreak, South Andaman.

INTRODUCTION

Dengue fever (DF) and dengue haemorrhagic fever (DHF) are mosquito-borne viral diseases of humans and are recognized as an important emerging public health problem throughout the world [1]. Epidemics of DF/DHF have occurred in tropical and subtropical countries, especially in the South East Asian region [2]. Dengue virus (DENV) is reported to be transmitted by *Aedes* mosquitoes, i.e. *Ae. aegypti* and *Ae. albopictus*. The infection is caused by any of

the four dengue virus serotypes (DENV-1,-2,-3,-4), which belong to the genus *Flavivirus*. This virus causes a spectrum of manifestations, ranging from DF to DHF and dengue shock syndrome (DSS), which is fatal and are usually associated with secondary infections [3]. Due to environmental changes and rapid urbanization, a considerable increase in the spread of dengue cases has occurred in the past four decades, in several parts of India. The disease has been found to occur both in urban as well as in rural areas [4].

Andaman & Nicobar Islands is an archipelago of more than 500 islands and islets, stretching over 700 km from north to south, in the Bay of Bengal. Serologically confirmed DF was first detected in these islands during 2009, although dengue antibodies were reported earlier [5]. There is the possibility that

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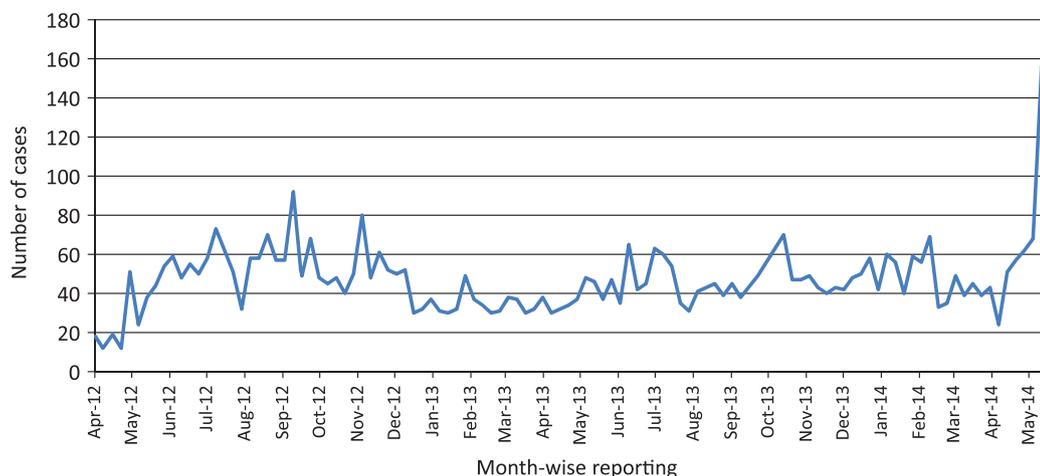


Fig. 1. Number of fever cases reported to Havelock Primary Health Centre during the week of reporting.

dengue viral infection has been occurring silently in Andaman & Nicobar Islands during the past few years, and there have been reports from the South Pacific Islands [6]. Our previous report suggested that DF is emerging as an important public health problem in these islands, due to the widespread prevalence of *Aedes* vector mosquitoes [7, 8]. In addition, presence of multiple serotypes of the virus, i.e. DENV-1, -2 and -3 in the islands elevates the risk of DHF and DSS and therefore, future dengue outbreaks could lead to higher morbidity and mortality [9]. An upsurge of fever cases of unknown origin, but resembling dengue and leptospirosis was reported in Havelock, Andaman & Nicobar Islands (an important tourism spot), during May–June 2014. Investigations were carried out to determine the aetiology, and the role of DENV, if any, during the outbreak.

MATERIALS AND METHODS

An upsurge in the number cases of febrile illness with symptoms similar to DF was observed during May 2014 at the Primary Health Centre (PHC) of Havelock, Andaman & Nicobar. Blood samples were collected from suspected patients and transported to the Regional Medical Research Centre (RMRC), Port Blair, to investigate and identify the aetiological agent.

All the samples were tested for the presence of anti-DENV, anti-chikungunya virus (CHIKV) IgM antibodies by IgM capture ELISA kits developed by the National Institute of Virology, Pune [10]. The samples were also tested for the presence of anti-leptospiral antibodies by microscopic agglutination

test (MAT) following standard procedures [11]. Reference strains belonging to 12 serogroups of *Leptospira*, common in the Andaman Islands and country, were included in the MAT panel as antigens. A subsample of the patients who reported to the hospital within 4 days of onset of symptoms and were negative for IgM anti-DENV antibodies was also tested for DENV RNA by reverse transcription–polymerase chain reaction (RT–PCR), followed by nested PCR, for detection of serotype. RNA was extracted from the serum samples using Qiagen Viral RNA extraction kit (Qiagen, USA) and RT–PCR was conducted following the standard protocol [12]. The first-round of PCR targeted a 511-bp fragment covering the capsid–protein C and pre-M regions of the viral genome, followed by nested multiplex PCR, to differentiate the serotypes of DENV, as the size of the amplified product was specific to each of the four serotypes. The PCR products were sequenced using the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems, USA) according to the manufacturer's instructions. All DNA sequences were assembled using SeqMan II v. 5.03 (DNASTAR, USA). These sequences were then aligned with previously published sequences of DENV strains belonging to various combinations of serotypes and genotypes by the ClustalW multiple alignment and pair-wise alignment program of MEGA software v. 5 [13]. Genetic distances were estimated using Kimura's two-parameter algorithm and a phylogenetic tree was constructed by the neighbour-joining method [13]. The statistical significance of the relationships obtained was estimated by bootstrap resampling analysis (1000 repetitions). In addition, the aligned DNA sequences

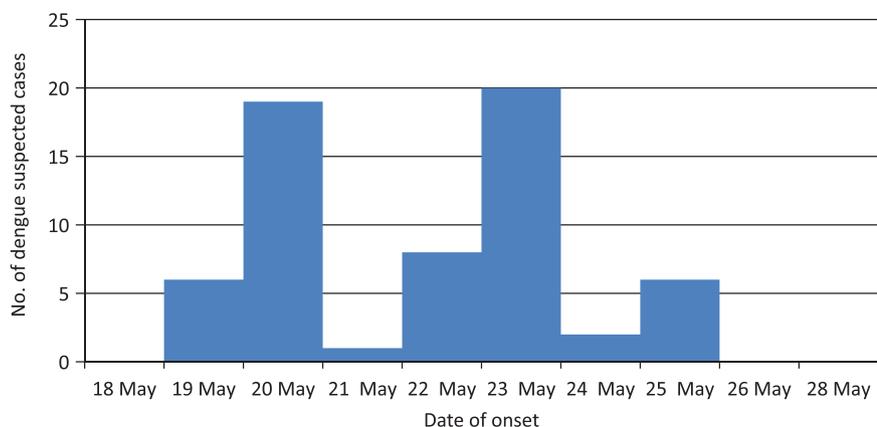


Fig. 2. Epidemic curve of dengue outbreak in Havelock, May 2014.



Fig. 3. Map of Havelock Island depicting distribution of positive cases.

were BLAST-searched to assess their identity with previously characterized sequences of the DENV genome.

The epidemic curve was prepared using the data on number of cases reported. Number of fever cases reported to PHC during the week of reporting in the previous 2 years was plotted to assess any increase

Table 1. Attack rates of dengue in villages of Havelock

Village	Population	Cases	Attack rate/1000
Havelock no. 1	857	10	11.7
Havelock no. 2	1257	9	7.2
Havelock no. 3	1279	24	18.8
Havelock no. 4	847	4	4.7
Havelock no. 5	463	2	4.3
Havelock no. 6	942	7	7.4
Havelock no. 7	351	1	2.8
Kalapathar	617	5	8.1
Total	6613	62	9.4

in fever cases beyond the expected occurrence. A spot map of cases was prepared using a copy of the map obtained from the Revenue Department. Attack rates in all the affected villages and for the whole island were estimated.

RESULTS AND DISCUSSION

Incidence of febrile illness in Havelock Island

A sudden upsurge of fever cases was reported at PHC, Havelock in May 2014. During the week commencing 12 May 2014, a total of 156 fever cases attended the PHC with headache, rigors, and pain in the joints, back muscles and abdomen. This is an unusual scenario in the small island where, the average number of cases reported per week over the last 2 years was 46.1 (95% confidence interval 19.4–72.9), which was clearly in excess of the expected number of fever cases (Fig. 1). In order to confirm the existence of febrile illness in Havelock, the Public Health Department of Andaman & Nicobar Administration requested the RMRC, Port Blair to conduct an

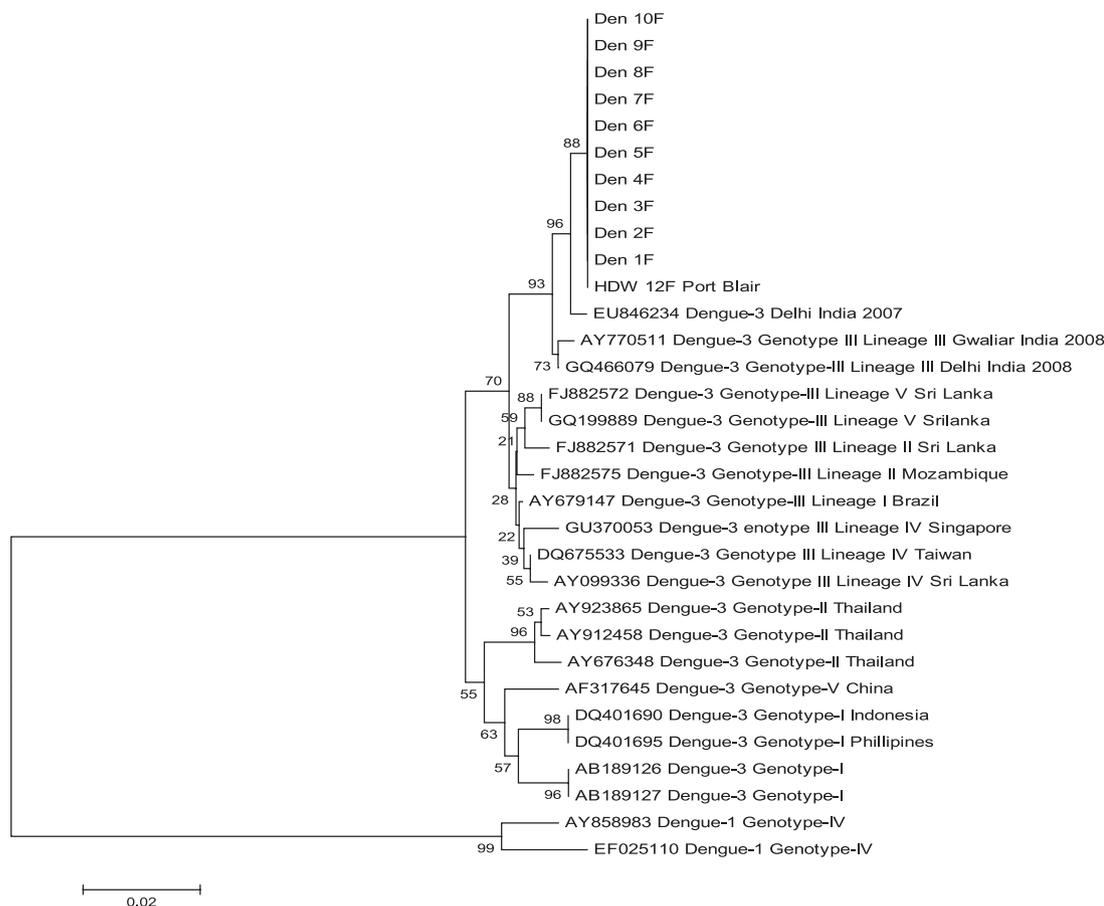


Fig. 4. Phylogenetic neighbour-joining tree showing 10 Havelock DENV sequences (Den 1F, Den 2F, Den 3F, Den 4F, Den 5F, Den 6F, Den 7F, Den 8F, Den 9F, Den 10F) that have been grouped with DENV serotype 3 and genotype III.

outbreak investigation in order to identify the infectious aetiology. A team comprising physician, laboratory technician and an entomologist visited the PHC and affected area between 15 May 2014 and 28 May 2014. Sixty-two suspected blood specimens were collected (on the basis of onset of symptoms, i.e. 1–5 days). Moreover, adult mosquito collection and *Aedes* larval infestation survey were conducted in the surrounding areas to confirm the transmission dynamics and the findings have been published recently [14].

Dengue had not been reported in Havelock until May 2014, although there was an initial peak of febrile illness on 12 May followed by a lull during 15–18 May and again a peak in 19–20 May 2014 which initiated a local outbreak in the village leading to the initial peak. The epidemic curve constructed from the data on suspected cases of dengue attending the PHC is shown in Figure 2. Later, due to the abundance of vector mosquitoes [14] resulting in the dissemination of the virus to other villages, a second peak occurred during

22–23 May. Simultaneously mosquito control measures were initiated by healthcare workers during this period and these clearly showed the subsidence of febrile cases by the end of May 2014.

Laboratory confirmation

Collected blood samples from the suspected patients attending the PHC and the household survey were screened for anti-DENV IgM antibodies by enzyme immunoassay (EIA) and two were found positive. All the samples were tested for DENV RNA, using RT-PCR and 25 were found positive. A total of 27 (43.5%) of 62 patients were diagnosed as having DENV infection, based on positive EIA or RT-PCR. There was no CHIKV IgM-positive and anti-leptospirosis antibodies detected in the samples collected. The results confirmed the DENV aetiology for the febrile illness, which occurred as an outbreak in Havelock Island during May 2014.

The total population of Havelock was 6613 and a total of 27 cases occurred during the outbreak (Fig. 3). Thus the attack rate was 9.4 cases/1000 population. The attack rates in villages are summarized in Table 1. The highest attack rate was observed in Havelock no. 3 village followed by Havelock no. 1. The nucleotide sequencing of the 511 bp amplicon confirmed that the virus sequence was homologous with DENV-3. Phylogenetic analysis of 10 strains from Havelock was compared with the sequences of representative strains of all serotypes of DENV. All the 10 capsid-protein C and pre-M region nucleotide sequences formed a single clade along with existing DENV serogroup 3 in Port Blair (Fig. 4). Dengue outbreaks with different serotypes have been reported in the mainland, India and also from Andaman & Nicobar Islands [9, 15]. Emergence of dengue serotype 3 in north and south India has been reported in the past [16, 17]. Earlier studies have reported the existence of two dengue serotypes, i.e. 1, 2 in these islands [9]. Recent investigations found the circulation of a third serotype of DENV in urban Port Blair during 2013 [8]. Interestingly, the recent entomological characteristic features indicate the existence of dengue serotype 3 during the outbreak in Havelock Island [14]. Therefore, the current findings envisaged that serotype 3 circulated in Havelock Island. According to antibody-dependent enhancement hypothesis, a secondary DENV infection could increase the risk factors for DHF/DSS [18]. Therefore, existence and circulation of multiple serotypes in these islands could precipitate the occurrence of DSS/DHF in the near future. Hence adequate precautionary measures need to be implemented both by the health authorities and the public to control the spread of dengue infection.

This investigation confirmed that the existence of an outbreak of febrile illness was due to DENV in Havelock during May 2014. The aetiological agent was identified as DENV serotype 3. This is the first outbreak of dengue reported from Havelock Island and the findings suggest that dengue, which appeared in Port Blair and adjoining areas a few years ago, is now spreading to other islands. DENV-3 infection was first identified in Andaman & Nicobar Islands in 2013 among wharf workers in Port Blair, South Andaman. Within a year, it has spread to Havelock Island, which is separated from South Andaman by about 36 nautical miles of open sea. As Havelock is an important tourist destination, frequent travellers between Port Blair and Havelock, particularly tourists, are likely to be infectious virus carriers to Havelock Island.

SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found at <https://doi.org/10.1017/S0950268816003423>.

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DECLARATION OF INTEREST

None.

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